Pharmaceutical Composition

BACKGROUND OF THE INVENTION

1. TECHNICAL FIELD

The invention relates to a pharmaceutical composition comprising certain oral available, LTB_4 antagonist, which contains a hydroxy and a benzamidine group, or a tautomer, a pharmaceutically acceptable salt, solvate, or physiologically functional derivative thereof (**1**) and at least one cyclooxygenase-2 inhibitor or a pharmaceutically acceptable salt, solvate, or physiologically functional derivative thereof (**2**), and a pharmaceutically acceptable carrier or excipient, and optionally one or more other therapeutic ingredients.

2. BACKGROUND INFORMATION

The US patent US 6,172,096 discloses a method of reducing recipient acute or chronic rejection of transplanted cells or organs, and for treatment of autoimmune diseases, hypersensitivity reactions of the acute or delayed type, allergic disorders, granulomas, meningitis, and septic shock by administering a cyclooxygenase-2 inhibitor and a leukotriene B_4 (LTB_4) receptor antagonist.

LTB_4 antagonists which contain a hydroxy and benzamidine group are compounds with pharmacologically valuable properties. Such LTB_4 antagonists may provide great therapeutic benefit, for example, in the treatment of arthritis, asthma, chronic obstructive lung diseases, psoriasis, ulcerative colitis, Alzheimer's disease, shock, reperfusion damage/ischaemia, cystic fibrosis, atherosclerosis and multiple sclerosis.

Such compounds are known e.g. from International Patent Applications WO 96/02497, WO 97/21670, WO 98/11062, WO 98/11119, WO 01/25186 and PCT/EP01/00262 .

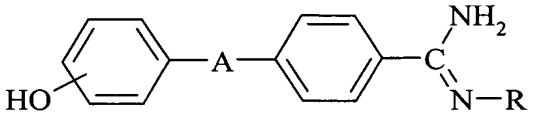
However, none of these prior art references indicates that the combination of a cyclooxygenase-2 inhibitor and a LTB_4 antagonists having a hydroxy and a benzamidine group will show a synergistic effect, in particular for the treatment of rheumatic arthtitis.

It has been demonstrated that arachidonic acid produced a dermal inflammation when applied topically (Carter et al, 5-Lipoxygenase inhibitory activity of zileuton. J Pharmacol Exp Ther 256, 929-937 (1990)). This inflammation is rich in neutrophils and consequently myeloperoxidase (MPO), a neutrophil marker enzyme, can be used as a quantitative index for cell infiltration. The mouse ear is especially suited to serve as a model of dermal inflammation induced by various agents like arachidonic acid which is known to be metabolized to several inflammatory mediators i.e. prostaglandines and LTB_4. Accordingly neither an LTB_4 antagonist nor an NSAID alone are supposed to fully counteract this kind of inflammation. Therefore this model seems to be useful to test the efficacy of a NSAID-LTB_4 antagonist combination .

It has now be found surprisingly that a pharmaceutical formulation comprising a cyclooxygenase-2 inhibitor and a LTB_4 antagonists having a hydroxy, preferably a phenolic hydroxy group and a benzamidine group shows a synergistic effect, in particular for the treatment of rheumatic arthtitis.

BRIEF SUMMARY OF THE INVENTION

The invention relates to a pharmaceutical composition comprising a LTB_4 antagonists having a hydroxy and a benzamidine group, preferably a compound of formula (I)

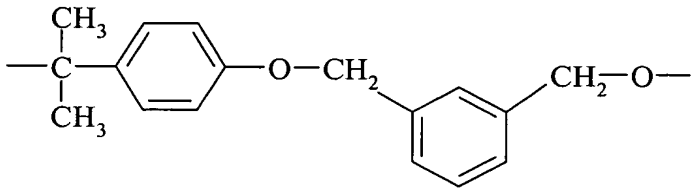


(I)

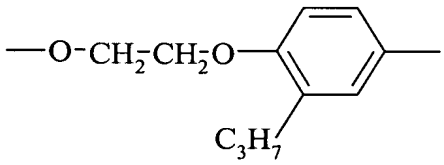
wherein

R represents a hydrogen atom or a group of formula $-CO_2-R'$, in which R' represents a C_{1-6} alkyl, an optionally substituted phenyl group or an optionally substituted benzyl group, wherein the optional substituents are selected from halogen atoms C_{1-6} alkyl, C_{1-6} alkoxy, cyano, nitro; C_{1-6} haloalkyl and C_{1-6} haloalkoxy groups, and

A is a group selected from the formulae (A1) and (A2):



(A1)



(A2)

or a tautomer, a pharmaceutically acceptable salt, solvate, or physiologically functional derivative thereof (**1**), and

at least one cyclooxygenase-2 inhibitor or combined cox1/cox2 inhibitor or a pharmaceutically acceptable salt, solvate, or physiologically functional derivative thereof (**2**), and a pharmaceutically acceptable carrier or excipient, and optionally one or more other therapeutic ingredients.

R preferably represents H or $-CO_2-C_2H_5$.

Another aspect of the present invention is a method for the prevention or treatment of a disease or disorder selected from the group consisting of arthritis, including rheumatoid arthritis, spondyloarthropathies, gouty arthritis, osteoarthritis, systemic lupus erythematosus and juvenile arthritis, asthma, hay fever, atopic dermatitis,rhinitis, bronchitis, COPD and cystic fibrosis, psoriasis, sclerodermia, morbus bechterew, sarcoidosis, tumor metastasis,morbus crohn, colitis ulcerosa, IBD, multiple sclerosis, arteriosclerosis, arteritis, myocardial infarction, stroke,

coronary heart disease which method comprises administration of effective amounts of a LTB_4 antagonist having a hydroxy and a benzamidine group, preferably a compound of formula (I) (**1**) and a cyclooxygenase-2 or combined cox1/cox2 inhibitor (**2**) to a patient in need thereof in a combined form, or separately or separately and sequentially wherein the sequential administration is close in time or remote in time.

Furthermore, the invention relates to the use of a LTB_4 antagonist having a hydroxy and a benzamidine group, preferably a compound of formula (I) (**1**) and a cyclooxygenase-2 inhibitor (**2**) in a combined form, or separately or separately and sequentially, wherein the sequential administration is close in time or remote in time, for the manufacture of a medicamentation for the prevention or treatment of disease or disorder selected from the group consisting of arthritis, including rheumatoid arthritis, spondyloarthropathies, gouty arthritis, osteoarthritis, systemic lupus erythematosus and juvenile arthritis, asthma, bronchitis, COPD and cystic fibrosis.

Finally, the invention relates to pharmaceutical kit comprising at least two separate unit dosage forms (A) and (B):

(A) one of which comprises a composition containing a LTB_4 antagonist having a hydroxy and a benzamidine group, preferably a compound of formula (I), a tautomer thereof or a pharmaceutically acceptable salt thereof (**1**), and optionally a pharmaceutically acceptable carrier;

(B) one of which comprises a composition containing one or more cyclooxygenase-2 inhibitors or combined cox1/ 2 inhibitors (**2**), and optionally a pharmaceutically acceptable carrier.

DETAILED DESCRIPTION OF THE INVENTION

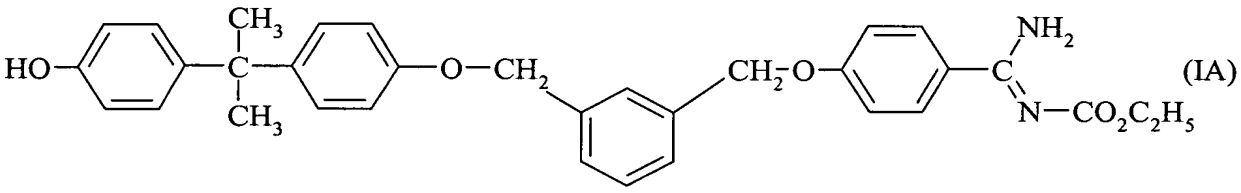
The term "LTB_4 antagonists which contain a hydroxy and benzamidine group" embraces compounds which selectively inhibit the leukotriene B_4 receptor and

corresponding produgs thereof. They have preferably a "rod-like" structure of up to 5, preferably 3 or 4 phenylene moieties, which are connected to each other by linking groups selected from single bonds, straight chained or branched C_{1-4}-alkylenediyl, -O-C_{1-4}-alkylenediyl, C_{1-4}-alkylenediyl-O- and -O-C_{1-4}-alkylenediyl-O-. One of the said phenylene moieties, preferably a terminal one, carries a amidine group (-C(=NH)-NH_2), wherein the imino hydrogen atom may also be replaced by a capping group which enhances the bioavailability of the compound and is cleaved of under physiological conditions. Preferably one of the othe phenylene moieties, most preferably the other terminal one, carries a phenolic hydroxy group.

The term "capping group" preferably represents a group of formula $-CO_2R'$, wherein R' has the meaning given hereinabove.

The term "C_{1-6} alkyl" embraces straight chained and branched alkyl groups having 1 to 6 carbon atoms such as methyl, ethyl *n*-propyl, *i*-propyl, *n*-butyl, 2-butyl, *n*-pentyl and *n*-hexyl.

Preferred is a pharmaceutical composition, wherein the active principle essentially consists of a compound of formula (I), in particular formula (IA)

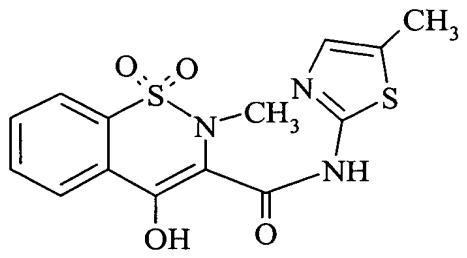


(IA)

(1) and one cyclooxygenase-2 inhibitor or combined cox1/cox2 inhibitor.

The term "cyclooxygenase-2 inhibitor" embraces compounds which selectively inhibit cyclooxygenase-2 over cyclooxygenase-1, or which are combined cyclooxygenase-1 and cyclooxygenase-2 inhibitors.

Preferred are the cyclooxygenase-2 inhibitor or combined cox1/coxII inhibitor selected from the group consisting of celecoxib, Dupont Dup 697, etodolac, etoricoxib, flosulide, meloxicam, nimesulide, parecoxib, rofecoxib, Taisho NS-398 and valdecoxib, in particular meloxicam of formula



or a pharmaceutically acceptable salt thereof.

The phrase "combination therapy" (or "co-therapy"), in defining use of a cyclooxygenase-2 inhibitor agent and a leukotriene B_4 receptor antagonist agent, is intended to embrace administration of each agent in a sequential manner in a regimen that will provide beneficial effects of the drug combination. The phrase also is intended to embrace co-administration of these agents in a substantially simultaneous manner, such as in a single capsule having a fixed ratio of these active agents or in multiple, separate capsules for each agent.

The phrase "therapeutically-effective" is intended to qualify the amount of each agent for use in the combination therapy which will achieve the goal of improvement in severity and the frequency of disease incidence over treatment of each agent by itself, while avoiding adverse side effects typically associated with alternative therapies.

The active substance of formula I may be present in the formulation according to the invention in the form of a physiologically acceptable acid addition salt. By physiologically acceptable acid addition salts are meant, according to the invention, pharmaceutically acceptable salts which are selected from the salts of hydrochloric acid, hydrobromic acid, sulphuric acid, phosphoric acid, methanesulphonic acid, acetic acid, fumaric acid, succinic acid, lactic acid, citric acid, tartaric acid and maleic acid. Mixtures

of the above acids may also be used to prepare the salts. According to the invention, the preferred salts of formula I are selected from among the hydrochloride, hydrobromide, sulphate, phosphate, fumarate and methanesulphonate. The salts selected from among the hydrochloride, hydrobromide and fumarate are particularly preferred. The active substance may optionally be in the form of a hydrate. Preferably, according to the invention, the compound of formula I is added to the tablet in the form of the free base and in the anhydrous form.

The pharmaceutical formulation according to the present invention is as a rule suitable for oral, intravascular, intraperitoneal, subcutaneous, intramuscular or topical administration, in particular oral administration.

The present invention preferably relates to a tablet containing a compound of formula I and a cyclooxygenase-2 inhibitor or a combined cox1/cox2 inhibitor which contains at least one pharmacologically acceptable excipient, and optionally at least one wetting agent.

The term "wetting agent" as used hereinbefore and hereinafter denotes an excipient which lowers the surface tension of water or other liquids so that they can penetrate into the surfaces of the tablets according to the invention and soak through them, displacing the air, thereby wetting them. The substances used as wetting agents are usually interface-active surfactants. These surfactants are amphiphilic (bifunctional) compounds with at least one hydrophobic and one hydrophilic part of the molecule. The hydrophobic group is usually a hydrocarbon chain, if possible a straight chain, with eight to 22 carbon atoms. Particular surfactants may also have (dimethyl)-siloxane chains or perfluorinated hydrocarbon chains as the hydrophobic part of the molecule. The hydrophilic group is either a negatively or positively charged (hydratable) or a neutral polar head group. Of the surfactants, anionic surfactants, particularly the long-chain alkylsulphates, especially sodium laurylsulphate and alkylbenzenesulphonates are preferred.

Within the scope of the present invention carbohydrates such as lactose or mannose, particularly finely divided lactose or sugar alcohols such as mannitol, sorbitol or xylitol, particularly mannitol, are of particular importance as excipients. These excipients have proved particularly advantageous in the formulation according to the invention. In a preferred aspect, therefore, the present invention relates to a tablet containing at least one compound of formula I, which contains, in addition to the active substance and the wetting agent, lactose, particularly finely divided lactose, more preferably lactose monohydrate or mannitol as excipient.

The tablet according to the invention may also contain compounds capable of acting as binders.

The term "binder" used hereinbefore and hereinafter denotes excipients which are suitable for binding other components to one another. Preferred binders according to the invention are selected from among:
powdered cellulose, microcrystalline cellulose, sorbitol, starch, polyvinylpyrrolidone (povidone), copolymers of vinylpyrrolidone with other vinyl derivatives (copovidone), cellulose derivatives, particularly methylhydroxypropylcellulose, e.g. Methocel A 15 LV, and mixtures of these compounds. The preferred binders are powdered cellulose, particularly microcrystalline cellulose and/or copovidone. Most preferred is a mixture of microcrystalline cellulose and a copolymer of vinylpyrrolidone and vinyl acetate, namely copovidone VA 64, the ratio of vinylpyrrolidone and vinyl acetate being about 3:2 (m/m). As a rule the tablet according to the invention has a weight ratio of microcrystalline cellulose to copovidone VA 64 of 20:1 to 1:1, preferably 15:1 to 2:1, particularly about 10:1 to 3:1. Thanks to this particularly preferred binder combination of microcrystalline cellulose and copovidone, tablets are obtained having a high bioavailability of the compounds of formula I.

The tablet according to the invention may also contain disintegrants in addition to the above mentioned ingredients. Within the scope of the present invention these disintegrants may optionally also be known as breakdown agents. These are preferably

selected, according to the invention, from among sodium starch glycolate, crosslinked polyvinylpyrrolidone (crospovidone), croscarmellose sodium salt (sodium salt of cellulose carboxymethyl ether, crosslinked), sodium-carboxymethylcellulose, dried maize starch and mixtures thereof. Within the scope of the present invention it is particularly preferred to use sodium starch glycolate, crospovidone and, preferably, the sodium salt of crospovidone or croscarmellose.

The tablet according to the invention may also contain flow agents or flow regulators and also lubricants, as additional ingredients. These include, within the scope of the present invention, for example, silicon dioxide, talc, stearic acid, sodium stearylfumarate, magnesium stearate and glycerol tribehenate. According to the invention magnesium stearate is preferably used.

In addition, the tablet according to the invention may contain one or more synthetic or natural, pharmaceutically acceptable dyes or colourings, preferably indigo carmine. If the abovementioned colourings are used the amount by weight thereof based on the total mass of the tablet according to the invention is 0.01 to 0.5 wt.%.

The active ingredients (**1**) and (**2**) are as a rule applied in a ratio, in which the resulting combination exhibits a synergistic effect. The term "synergistic effect" as used herein relates to an effect, which is higher than tone could expect from the additive effects of each single active ingredient.

Accordingly, the pharmaceutical formulation according to the present invention exhibits as a rule (**1**) and (**2**)in synergistically effective amounts of, preferably a weight ratio of (**1**) to (**2**), which ranges from 50 : 1 to 1 : 300, preferably from 8:1 to 1:80, in particular 1:1 to 1:40, most preferably 1:5 to 1:30.

The pharmaceutical formulation according to the present invention are preferably administered in a single application dose containing 1 to 10,000 milligrams, preferably 5

to 1000 mg of the combined active ingredients (**1**) and (**2**). Most preferred is a formulation comprising about 10 mg meloxicam and up to 300 mg of formula IA.

The Examples that follow serve to illustrate some formulations according to the invention. They are intended solely as possible procedures described by way of example, without restricting the invention to their content.

Example 1

Ingredients	mg/tablet
(01) compound IA, jet-ground	1,000
(02) meloxicam	10,000
(03) microcrystalline cellulose	15,000
(04) mannitol	52,250
(05) croscarmellose sodium	1,500
(06) sodium laurylsulphate	0,050
(07) indigo carmine (11-14 %)	0,075
(08) magnesium stearate	1,125
	81,000

The direct compression comprises producing a premix of ingredients (01), (02), (06), (07) and some of (04) with an intensive mixer. The premix is screened and mixed with ingredients (03), (05) and the remainder of (04) in a gravity mixer. After the mixture has been screened again, ingredient (08) is added.

Example 2

2.1 Animals

Female albino mice (Han:NMRI) obtained from Interfauna and weighing about 20-25 g were used. The animals were provided with standardised pellet diet (Altromin 8013)

and had tap water freely available. The animals were accommodated in a climatized room with a 12 hours light/dark cycle and kept in groups.

2.2 Chemical substances

Compound of formula IA:

Carbamic acid, [[4-[[3-[[4-[1-(4-hydroxyphenyl)-1-methylethyl]-phenoxy]methyl]phenyl]methoxy]phenyl]iminomethyl]-, ethyl ester was prepared as described in US 6,417,382.

Meloxicam was provided by Boehringer Ingelheim Pharma KG

Arachidonic acid was purchased from Sigma (A9798) and dissolved 1:10 in acetone.

2.3 Study Design

The compound of formula IA and meloxicam were administered orally (0.2 ml / 10 g bw) 30 min. before arachidonic acid challenge. Meloxicam was given twice: one dose 16 hours and the second dose 30 minutes before challenge. For every day there was a concurrent control. Then number of animals per group was 7. The study compounds were suspended in 1% methylcellulose (Tylose MH 300, Fluka,CH-9470 Buchs). The experiment was run in five groups. Details are given in Table 1. Every group contained one control, two doses of meloxicam, two doses of (IA), and one dose of the combination of the two compounds.

Table 1

Treatment	N	Dose m/kg p.o.) Group 1	Dose m/kg p.o.) Group 2	Dose m/kg p.o.) Group 3	Dose m/kg p.o.) Group 4	Dose m/kg p.o.) Group 5
Control (Tylose)	7	-	-	-	-	-
Meloxicam	7	1	2	4	8	16

Treatment	N	Dose m/kg p.o.) Group 1	Dose m/kg p.o.) Group 2	Dose m/kg p.o.) Group 3	Dose m/kg p.o.) Group 4	Dose m/kg p.o.) Group 5
Meloxicam	7	2	4	8	16	32
(IA)	7	0.05	0.1	0.2	0.4	0.8
(IA)	7	0.1	0.2	0.4	0.8	1.6
Meloxicam		1	2	4	8	16
plus (IA)	7	0.05	0.1	0.2	0.4	0.8

2.4 Experimental Procedure

Mice were lightly anesthetized by ether and 1 mg arachidonic acid (10 µl) was applied to each side of the left ear. The right ear remained untreated, acetone alone did not cause any late response. The animals were sacrificed by ether 6 hours later, and a biopsy (diameter 8 mm) was punched out from both ears to assess an increase of neutrophils in the left ear compared with the right ear. Tissue samples were homogenized in 1 ml 0.5% HTAB (Hexadecyl-trimethyl-ammonium-bromide; Sigma H-5882; solved in 0.05 M phosphate buffer, pH 6.0) using a tissue homogenizer (IKA-Ultraturrax T5; Janke & Kunkel, Staufen/Breisgau) at 30000 RPM for 15 seconds. After centrifugation (16000 G, 5 min) the supernatants were frozen until processing for myeloperoxidase (MPO). Determination in the supernatants for MPO, a neutrophil marker enzyme, served as a quantitative index for the neutrophil accumulation.

MPO was determined spectrophotometrically at 450 nm using a micro plate version of the method of Bradley (1982) and a micro plate reader (V_{max}; Molecular Devices, Palo Alto) suitable for kinetic measurements and expressed as mean optical density per minute.

2.5 Statistical Evaluation

For each individual the response myeloperoxidase activity (MPO) in optical density per minute (oD/min) was measured.

Based on these results the following statistical analyses were done:

- Comparison of control groups
- Comparison of Control vs. Treatments separately for each group
- Comparison of high vs. low dose for both treatments separately for each group
- Comparison of combined treatment vs. high doses separately for each group
- Comparison of combined treatment vs. other treatments in group 5

For the comparisons the nonparametric statistical methods Kruskal-Wallis-One-Way-ANOVA and the Wilcoxon-Two-Sample-Test (2sided) have been used. For the first and last comparisons also Bonferroni-Holm adjusted p-values have been calculated.

The calculations and statistical analysis were done with the NPAR1WAY procedure of the SAS software program (SAS Institute Inc., Cary, North Carolina) version 8.2. (IA) and meloxicam both inhibited arachidonic acid induced ear inflammation in mice. Details of statistical analysis are shown in tables 2 to 4.

Table 2 Difference between treatment and control

Group	**Treatment** Compound / dose [mg/kg]	**p-value**	**p-value (adj)**
1	Meloxicam / 1	1.0000	n.s.
	Meloxicam / 2	0.2502	n.s.
	(IA) / 0.05	0.7983	n.s.
	(IA) / 0.	0.7491	n.s.
	Meloxicam / 1+(IA) / 0.05	0.8983	n.s.
2	Meloxicam / 2	0.0152	0.0304
	Meloxicam / 4	0.1252	0.1252

Group	Treatment Compound / dose [mg/kg]	p-value	p-value (adj)
	(IA) / 0.1	0.0060	0.0240
	(IA) / 0.2	0.0073	0.0219
	Meloxicam / 2 + (IA) / 0.1	0.0022	0.0110
3	Meloxicam / 4	0.0736	0.0736
	Meloxicam / 8	0.0049	0.0147
	(IA) / 0.2	0.0073	0.0146
	(IA) / 0.4	0.0033	0.0165
	Meloxicam / 4 + (IA) / 0.2	0.0033	0.0165
4	Meloxicam / 8	0.3067	n.s.
	Meloxicam / 16	0.7015	n.s.
	(IA) / 0.4	0.0553	0.1659
	(IA) / 0.8	0.0106	0.0424
	Meloxicam / 8 + (IA) / 0.4	0.0022	0.0110
5	Meloxicam / 16	0.1599	n.s.
	Meloxicam / 32	0.2246	n.s.
	(IA) / 0.8	0.0049	0.0196
	(IA) / 1.6	0.0072	0.0216
	Meloxicam / 16 + (IA) / 0.8	0.0017	0.0085

Table 3

Comparisons High/Low Treatment and High Treatment vs Combination

Group	Low Dose [mg/kg]	High Dose [mg/kg]	p-value
1	Meloxicam /1	Meloxicam / 2	0.3067
	(IA) / 0.05	(IA) / 0.1	0.8983
2	Meloxicam / 2	Meloxicam / 4	0.7983
	(IA) / 0.1	(IA) / 0.2	0.7983

Group	Low Dose [mg/kg]	High Dose [mg/kg]	p-value
3	Meloxicam / 4	Meloxicam / 8	0.0474
	(IA) / 0.2	(IA) / 0.4	0.5229
4	Meloxicam / 8	Meloxicam / 16	0.7983
	(IA) / 0.4	(IA) / 0.8	0.9490
5	Meloxicam / 16	Meloxicam / 32	0.4320
	(IA) / 0.8	(IA) / 1.6	0.7012

Group	High Dose [mg/kg]	Combination[mg/kg]	p-value
1	Meloxicam / 2	Meloxicam / 1 + (IA) / 0.05	0.1792
	(IA) / 0.1	Meloxicam / 1 + (IA) / 0.05	0.8983
2	Meloxicam / 4	Meloxicam / 2 + (IA) / 0.1	0.0033
	(IA) / 0.2	Meloxicam / 2 + (IA) / 0.1	0.0348
3	Meloxicam / 8	Meloxicam /4 + (IA) / 0.2	1.0000
	(IA) / 0.4	Meloxicam / 4 + (IA)/0.2	0.3067
4	Meloxicam / 16	Meloxicam / 8 + (IA) / 0.4	0.0033
	(IA) / 0.8	Meloxicam /8 +(IA) / 0.4	0.0215
5	Meloxicam / 32	Meloxicam / 16 + (IA) / 0.8	0.0026
	(IA) / 1.6	Meloxicam /16 + (IA) / 0.8	0.0086

Table 4

Comparisons Combination vs Others for Group 5

Comparison vs combined (Group5)	p-value	p-value (adj)
Control	0.0017	0.0085
Meloxicam / 16	0.0032	0.0096
Meloxicam / 32	0.0026	0.0104
(IA) / 0,8	0.0090	0.0090

Comparison vs combined (Group5)	p-value	p-value (adj)
(IA) / 1,6	0.0086	0.0172

The arachidonic acid induced mouse ear inflammation test is indicative for pathological processes where neutrophils are involved (see also introduction). It shed light especially on the chemoattractant part of the arachidonic acid cascade induced neutrophil activation. The results indicate that (IA) and Meloxicam are effective orally and consequently may target important parts of this inflammation..

With respect to the combination of both compounds, there are two criteria which must be fulfilled to prove a super-additive efficacy (potentiation).

i.) the maximal achievable effect of the combination must be bigger than the maximal achievable effect of the single compound.

ii.) the effect of the combination should be bigger than the effect which can be expected from the dose response curve of the single compounds

The first criterion is experimentally tested by using supra-maximum doses of the single compounds and compare these with the achievable maximal effect of the combination. This may be difficult when the compounds under investigation produce a 100% inhibition itself. Therefore the model of arachidonic acid induced mouse ear inflammation was chosen, because NSAIDs and LTB_4 antagonists demonstrate very shallow dose response curves and a 100% inhibitory effect is hardly possible. As shown in the present experiments of group5 the supra-maximum doses of meloxicam (16 and 32 mg/kg p.o.) and (IA) (0.8 and 1.6 mg/kg p.o.) achieved maximum inhibitory effects o 37% and 66% respectively, whereas the combination (meloxicam 16 mg/kg p.o., (IA) 0.8mg/kg p.o.) achieved a maximum inhibition of 96%.This difference was statistically significant and consequently proves a super-additive effect according to criterion i.).

The second criterion can be tested by doubling the doses of the single compounds and compare the effect of the higher doses of the single compounds with the effect of the

combination of the lower doses of the single compounds. In group 1 all doses were too low to achieve any effect and consequently the results of this experiment cannot be used either to accept or reject the hypothesis of potentiation. The same holds true for the experiments in group 3. Although the combination achieved the highest inhibition, the difference to the inhibition reached with the highest doses of the single compounds were not statistically significant, because the single compounds alone caused already high inhibition values. However the experiments performed in group 2, 4, and 5 clearly show that the combination was significantly more effective than the higher doses of the single compounds thus proving a super-additive efficacy according to criterion i.i.

It is concluded that the combination of the non steroidal anti inflammatory drug meloxicam with the LTB_4 antagonist (IA) strongly inhibits arachidonic acid induced mouse ear inflammation after oral administration in an super-additive way